

May 09, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJG_2837_editid_2013_05_09.doc).

Title: Effect of DA-9701 on gastric emptying in a mouse model: Assessment by ^{13}C -octanoic acid breath test

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

It remains unclear how helpful the animal model is in reflecting human conditions and correcting pathologies.

→ We agree with your opinion. As we mentioned in the discussion, a previous study reported that DA-9701 showed accelerated gastric emptying in a model of delayed gastric emptying induced with apomorphine and cisplatin. We are planning a study with a gastroparesis model in streptozocin induced-diabetes mouse using the ^{13}C -octanoic acid breath test.

The m-value which gives the percentage of the cumulative tracer recovery in indefinite time appears high in the mice, around 75% which is about double of what we would expect in human studies. Can you comment on this?

→ Thank you for kind comment. We considered that there may be differences among the species. The range of m-value in this study was about 60~85%. Although we could not find reference data about the m-value in a mouse, the m-value measure in a rat was reported to be about 60~70% (Neurogastroenterol. Mot. 2002;14:287-293).

What was the atom percent excess of the labeled ^{13}C -octanoic acid?

→ Thank you for pointing this out. We used Octanoic acid-1- ^{13}C , 99 atom % ^{13}C . We added it in the

method. (page 5 line 19)

Are there any human studies underway or planned?

→ A human study including gastric emptying, gastric accommodation, small bowel transit, and colon transit is currently underway.

Abstract: It is important to make a modification of the conclusions; which should say the aspects more related to the experiment made for the researchers.

→ We agree with your opinion. We would like to add the usefulness of the breath test in the conclusion of the abstract. However, WRITING REQUIREMENTS OF BRIEF ARTICLES of WJG state that the conclusion be no more than 26 words. As such, we could not include this aspect in our conclusion.

Introduction: The introduction describes, explains and predicts the study problem; however, it is advisable to present more citations from scientific papers to explain the pharmacokinetics and pharmacodynamics of DA-9701.

→ Thank you for your opinion. We added it in the introduction as you recommended. However, publications about DA-9701 are few, limiting the references inserted.

“DA-9701 and its components accelerated gastric emptying and improved gastric accommodation in animal model.” (page 4 line 5-6)

Methodology: Further explanation why the authors choose erythromycin, as an agent to compare the effects of DA-970.

→ Thank you for your kind comment. Erythromycin was chosen as the positive control because it is a well known gastrokinetic agent and used in the treatment of gastroparesis.

In the statistical analysis of data, it is advisable the authors perform the following steps:

1. To determine differences pre-post treatment (DA-9701, erythromycin and saline) in gastric emptying times [the half time ($T_{1/2}$), the lag phase for 10% emptying (T_{lag10}), and the lag phase for 15% emptying (T_{lag15})].
2. To compare the differences described above in point 1, using rank Kruskal Wallis test. It is advisable to use rank Kruskal-Wallis test; to compare the effects of the drug treatments on gastric emptying the

cumulative excretion curve for ^{13}C -octanoic acid breath showed accelerated gastric emptying after treatment with DA-9701 compared with that after treatment with saline and with that of erythromycin.

→ Thank you for your kind opinion. In our study, 10 mice had two tests to test reproducibility and another 12 mice had 3 tests. We performed repeated breath tests in same animal with positive, negative control, and placebo. We considered that this cross-over experiment could reflect the gastrodukinetic effects of DA-9701 and the saline treatment can be considered as pretreatment. We used the Wilcoxon signed-rank test for comparisons because the half gastric emptying times and lag phases after drug treatment are numerical data in related groups. We additionally performed a repeated measurements ANOVA for comparisons of cumulative excretion curve at every 60 min points and significant difference between DA-9701 group and saline group was revealed. We added it in to the manuscript. (page 7 line 17-19, page 8 line 7-10)

Results: Avoid repeating the results already presented in figures and tables. Instead better highlight the most important results found by the authors.

→ Thank you. We have attempted to change our manuscript per your suggestion.

Explain if all animals that began the study completed it. Also explain the adverse effects or undesirable with DA-9701 or erythromycin

→ All animals that began the study completed it. There was no detectable adverse effect with DA-9701 or erythromycin in mice.

In Table 1, show the number of animals per group.

→ Thank you. We added it to the table

Conclusions: It is important to make a modification of the conclusions; which should say the aspects more related to the experiment made for the researchers.

→ Thank you for your opinion. We added the following to the conclusion. "The ^{13}C -octanoic acid breath test can be a useful tool to reflect physiological or pharmacological effects on gastric motility, especially in developmental programs for new prokinetic drugs." (page 10 line 27-29)

One conceptual remark is about the lack of a pathological group of animals with a delay in gastric

emptying. For example the authors are encouraged to test DA-9701 in mice prone to or with diabetes mellitus, a condition known to affect gastric emptying in humans as well as in animals. Indeed, going back to the good old times when erythromycin was for the first time used in the clinical setting, that drug was tested in diabetic patients with gastroparesis.

→ We agree with your opinion. As we mentioned in the discussion, a previous study reported that DA-9701 showed accelerated gastric emptying in a model of delayed gastric emptying induced with apomorphine and cisplatin. We are planning a study with a gastroparesis model in streptozocin induced-diabetes mouse using the ^{13}C -octanoic acid breath test.

Another issue pertains to the methodology, and the normalization of the data vs saline in such a way to minimize the biological variability evident in Figure 4A and 4B.

→ Thank you for your kind comment. We intended to show the intra-individual differences evoked by drug treatment. We had tried other methods of presenting the data. However, the other methods could not convey our meaning as clearly and despite the biologic variability in Figure 4A & B, it is the authors' belief that this method is the clearest one.

Furthermore, from the emptying curves it is unclear what corresponds to a "lag-phase" typical of emptying of solids.

→ Thank you for your kind comments. We added it to the figure.

The paper needs a thorough editing for the English grammar. There are also typos throughout the text.

→ Thank you for your comment. English language editing was performed by professional English editing company.

What is the hypothesis of this study?

→ Thank you. We hypothesized that the gastroduodenal effects of DA-9701 could be demonstrated in the same animal by repeated ^{13}C -octanoic acid breath test in mouse model.

It appears that 12 mice had 3 tests, and a subset, 10 mice had an additional test to offer reproducibility. Were 2 mice excluded?

→ Thank you. 10 mice were used to test reproducibility and another 12 mice had 3 tests. We revised

this in material and methods.

The authors obtained breath samples at 15 minutes intervals, but the difference in median 1/2 gastric emptying time between DA-9701 and saline controls is 12 min. Is this difference significant?

→ Thank you for your kind opinion. Twelve min of the difference in median 1/2 gastric emptying time between DA-9701 and saline controls might be ambiguous. The mean 1/2 gastric emptying time of DA-9701 and saline controls are 122 min and 148 min. Our study was focused at the intra-individual difference of drug treatment and significant difference was showed by the Wilcoxon signed-rank test.

How does the potential of DA-9701 relate to the potency of 5-HT₄ receptor agonists?

→ Thank you. In vitro and in vivo preliminary pharmacological studies of DA-9701 have demonstrated that DA-9701 has a moderate affinity for the 5-HT₄ receptors with agonistic effects on 5-HT₄ receptor.

Could the authors please specify why they used a non-parametric analysis instead of a repeated measurements ANOVA? Please specify whether the statistical analysis indeed analysed the data of the three measurements points in the first time followed by posthoc testing.

→ Thank you for your kind opinion. We used the Wilcoxon signed-rank test for comparisons because the half gastric emptying times and lag phases after drug treatment are numerical data in related groups and the number of mice used in this study are not so many. We did not initially consider using repeated measurements ANOVA for statistical analysis. We performed a repeated measurements ANOVA for comparison of half gastric emptying times and lag phases as your recommendation and the differences were not significant among the groups. We also performed a repeated measurements ANOVA followed by post hoc testing for comparison of total cumulative excretion curve at the point of every 60 min and there was a significant difference between DA-9701 and saline treatment. We added the method and results of a repeated measurements ANOVA for comparison of total cumulative excretion curve to the manuscript. (page 7 line 17-19, page 8 line 7-10)

All data are presented in the results section and in the tables and figures repeating the same information.

→ Thank you. We have attempted to change our manuscript per your suggestion.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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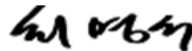
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